Supporting Information

Parthenolide inhibits ubiquitin-specific peptidase 7 (USP7), Wnt signaling, and colorectal cancer cell growth

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Inventory of Supporting Information:

Figure S1

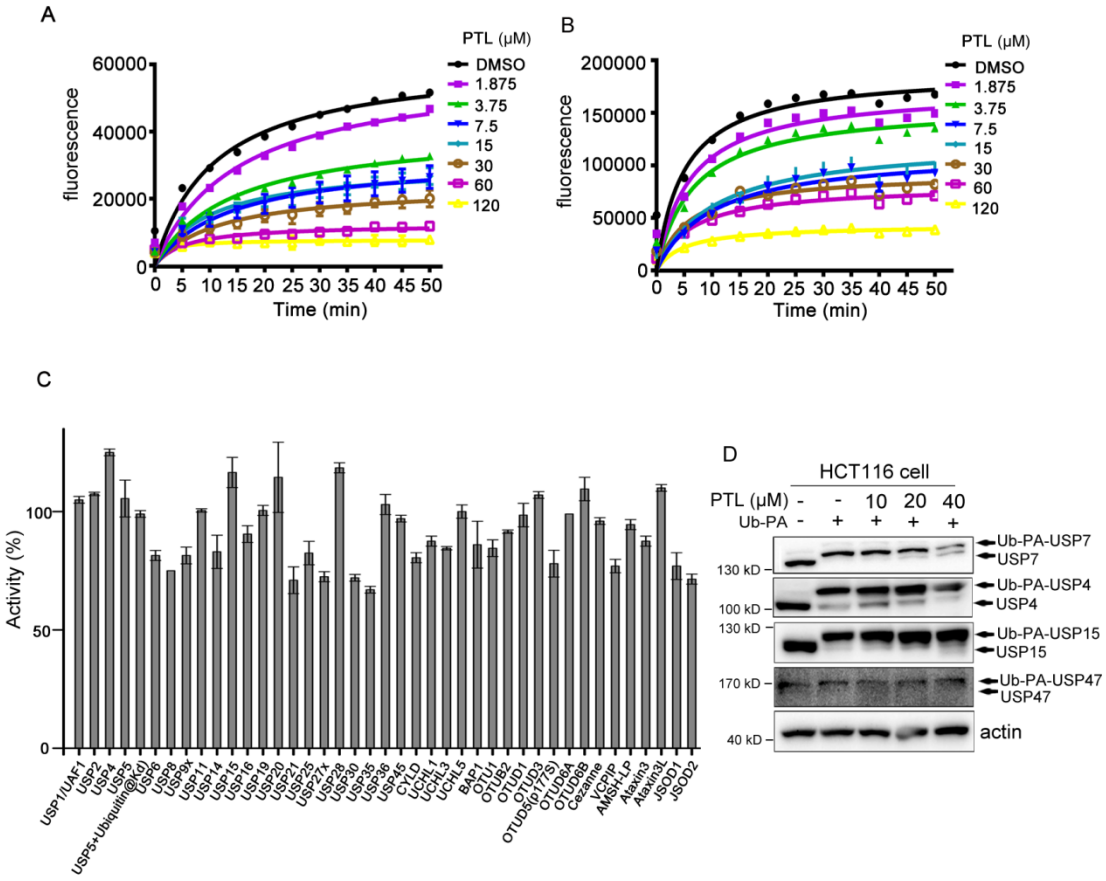
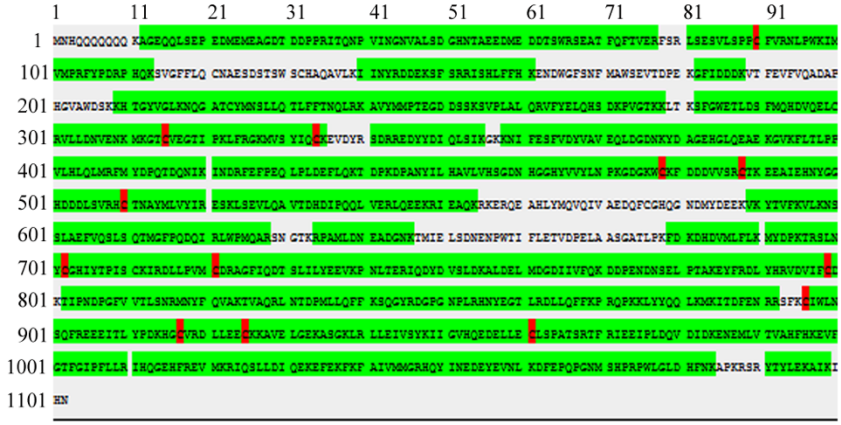


Figure. S1 Identification of PTL as a USP7 inhibitor. (A) PTL inhibited USP7 enzymatic activity in a concentration dependent manner in the Ub-AMC hydrolysis assay. (B) PTL inhibited USP7 enzymatic activity in a concentration dependent manner in the Ub-R110 hydrolysis assay. (C) Selectivity profile of PTL against a panel of DUBs. Screening was performed at a fixed concentration of 100 μM PTL by Ubiquigent. Data were presented as mean ± SD (n=2). (D) HCT116 cells were treated with indicated doses of PTL for 2 h and then labeled with Ub-PA. Individual DUBs were detected using specific antibodies.

Figure S2

A



B

Sites of PTL modification on recombinant USP7

adduction sites on USP7	peptide sequence
Cys90	LSESVLSPPC*FVR
Cys315	MKGTC*VEGTIPK
Cys334	MVSYIQC*K
Cys478	WCKFDDDVVSR
Cys488	C*TKEEAIEHNYGGHDDDLSVR
Cys510	HCTNAYM°LVYIR
Cys702	SLNYC*GHIYTPISCK
Cys721	DLLPVM°C*DR
Cys799	VDVIFC*DK
Cys896	C*IWLNSQFR
Cys917	EEEITLYPDKHGC*VR
Cys925	DLLEEC*KK
Cys961	IIGVHQEDELLEC*LSPATSR

*Indicates modified residue

°Oxidation of some amino acids

Figure. S2 Determination of PTL binding sites in USP7 using LC-MS/MS. 3 μg recombinant USP7 protein were incubated with 50 μM PTL at room temperature for 2 h, and then separated by SDS-PAGE and visualized by coomassie brilliant blue. The protein-containing bands in gel were excised, followed by in-gel digestion and analyzed by LC-MS/MS on Nano LC-LTQ-Orbitrap XL (Thermo Finnigan, San Jose,

CA). Data processing was done using Proteome Discoverer 1.4 software. (A) Sequence coverage achieved in MS peptide mapping experiments: covered sequence (84%) is green-highlighted and ligand-bound cysteines are red-highlighted. (B) The cysteines and corresponding peptides covalently targeted by PTL in MS experiments.